Table 1

Examples of Autoimmune Diseaes or Disease Model Caused By Autoreactive B Cell Responses

Disease	Pathogenic Antibody Specificity
Myasthenia Gravis (MG)	Anti-acetylcholine receptor antibodies cause weakness in MG
Juvenile Onset Diabetes Mellitus (Type 1 Diabetes)	Anti-insulin antibodies and anti-islet cell antibodies mediate islet cell destruction
Graves' Disease	Anti-thyroid stimulating hormone receptor antibodies mediate the disease
Insulin Resistance in Diabetes Mellitus	Anti-insulin antibodies prevent treatment of diabetes with insulin

Table 2

Examples Autoimmuine Diseases or Disease Models Caused by Autoreactive T Cell Responses

Experimental autoimmune uveoretinitis (EDU)	T cell responses against retinal S antigen cause eye damage
Experimental autoimmune encephalomyelitis (EAE)	T cell responses against myelin basic protein cause neuronal damage

Table 3

Peptide Sequences Used In Chimpanzee Immunizations

F-T1-SP10IIIB(A)	AVGIGALFLGFLKQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPGRAFVTI
T1-SP10IIIB	KQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPG
T1SP10IIIB(A)	KQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPGRAFVTI

Table 4

Tritiated Thymidine Incorporation of Peripheral Blood Mononuclear Cells Following In Vitro Stimulation With HIV Env gp120*

Chimpanzee No.	Immunogen	Pre-Immunization	Post-Immunization
		Δcpm/10^6 cells (Post/Pre)	
884	T1-SP10IIIB, then T1-SP10IIIB(A)	169	39,189 (232)
1028	T1-SP10IIIB, then T1-SP10IIIB(A)	17,955	129,121 (7)
1045	F-T1-SP10IIIB(A)	6,348	12,256 (2)
1070	F-T1-SP10IIIB(A)	11,285	22,719 (2)

*Data represent the peak gp120 responses observed during the immunization period. Data for animals 884,1028, and 1045 represent peak responses using from 2ug/ml to 0.5ug/ml of HIVIIIB(LAI) recombinant gp120. Data for animal 1070 represent peak responses using from 1ug/ml to 0.5ug/ml of native HIVIIIB(LAI) gp120.

Table 5

HIV Envelope gp41 Fusion Protein (F) Sequences From Multiple HIV Isolates

Isolate	Sequence
HIV-1	
BH10	A V G : I G A L F L G F L
MN	A A : : - - - - - - - - -
SC	- - - T - - - M - - - - -
SF2	- - - I V - - M - - - - -
CDC4	- - - M L - - M - - - - -
WMJ2	- - - T - - - M - - - - -
RF	- - - T - - - M - - - - -
ELI	- - I - : L - - M - - - - -
MAL	- - I - : L - - M - - - - -
Z6	- - I - : L - - M - - - - -
Z321	- - I - M : - - F - - - - -
JY1	- - I - - : L - - V - - - - -
WMJ-1	- - - A - - - M - - - - -
HIV-2	
ROD	R G V F V L G F L G F L
NIHZ	- - - - - - - - - - - -

Sequences for BH10 are aa 519-530 from Ratner, L, et al. Nature 313: 277-284, 1985. Sequences for the remainder of the HIV-1 and HIV-2 isolates from Myers, et al. Human Retroviruses and AIDS, 1988, Los Alamos National Laboratory, Los Alamos, New Mexico, p. II-90. WMJ-1 sequence from ref. 18.

Table 6

Regions of the TSH Receptor to Which Patient Anti-TSH Receptor Autoantibodies Bind

Amino Acid No.	Sequence	Ref.
333-343	YVFFEEQEDEI	17
12-36	HQEEDFRVTCKDIQRIPSLPPSTQT	18
289-317	LRQRKSVNALNSPLHQEYEENLGDSIVGY	18
352-366	YYVFFEEQEDEIIGF	27
103-111	YKELPLLKFL	28

Amino acid numbers and sequence from the reference listed

Table 7

Examples of Hybrid Peptide Constructs That Could Be Used To Treat Anti-HLA Immune Responses In AIDS

HIV gp120 homology with DP/DQ β chain

gp120 aa261-270	VVSTQLLLNG
HLA DP/DQ aa142-151	VVST*LI*NG

HIV gp41 homology with HLA DR β chain

gp41 aa837-844	EGTDRVI
HLA DR aa19-25	NGTERVR

Hybrid Immunogens: AVGIGALFLGFLVVSTQLLLNG
AVGIGALFLGFLVVSTLING
AVGIGALFLGFLEGTDRVI
AVGIGALFLGFLNGTERVR

HIV gp120 and gp41 homologies with HLA Class II are from refs. 25 and 26.

TABLE 8

Sequences of Synthetic Peptide Constructs
Derived From HIV MN and HIVIIIB Env gp120*

Peptide Name	Peptide Type	Peptide Composition and Sequence (Epitope Type)			
		F	T1(Th)	SP10(B cell)	A(B cell)
F-T1-SP10IIIB(A)	F-Th-B	AVGIGALFLGFL	KQIINMWQEVGKAMYA	CTRPNNNTRKSIRIQRGPGRAFVTI	
T1-SP10IIIB(A)	Th-B		KQIINMWQEVGKAMYA	CTRPNNNTRKSIRIQRGPGRAFVTI	
T1-SP10IIIB	Th-B		KQIINMWQEVGKAMYA	CTRPNNNTRKRIRIQRGPG	
T1-SP10MN(A)	Th-B		KQIINMWQEVGKAMYA	CTRPNYNKRKRIHIGPGRAFYTTK	

*Each amino acid is represented by a single-letter code that is the first letter of its name, except for arginine (R), asparagine (N), glutamine (Q), glutamic acid (E), lysine (K), phenylalanine (F), tryptophan (W), tyrosine (Y), and aspartic acid (D). F (fusogenic domain) sequence is amino acids 519-530 from HIVIIIB (27). T1 sequence is amino acids 428-443 from HIVIIIB (27). SP10MN(A) sequence is amino acids 301-319 from HIVMN (28). SP10IIIB sequence is amino acids 303-321 from HIVIIIB. (A) sequence is amino acids 320-324 from HIVMN (28) and amino acids 322-327 from HIVIIIB (27).

Th= T helper cell determinant.
B cell = B cell neutralizing antibody determinant.
A = Additional HIV gp120 V3 loop sequences added to the original synthetic peptide (SP10) sequence to add an additional neutralizing and CTL region to the HIV B cell determinant of the hybrid peptide.

27 = Ratner et al, Nature 313:277 (1985)
28 = Myers et al, Human Retroviruses and AIDS (1991), p. III 6-23

Table 4

Time Course of Anti-Peptide Antibody Responses in Chimpanzees Immunized with HIV Envelope Synthetic Th-B or F-Th-B Peptides

Month of Study	Immunogen	Chimpanzee Number 884 Reciprocal of ELISA Titer	Chimpanzee Number 1028 Reciprocal of ELISA Titer	Immunogen	Chimpanzee Number 1045 Reciprocal of ELISA Titer	Chimpanzee Number 1070 Reciprocal of ELISA Titer
1		0	0		0	0
2	Th-B(IIIB) 6mg	0	0	F-Th-B(IIIB) 6mg	0	0
3	Th-B(IIIB) 6mg	51,200	102,400	F-Th-B(IIIB) 6mg	0	0
4	Th-B(IIIB) 6mg	25,600	819,200	F-Th-B(IIIB) 6mg	0	800
5	Th-B(IIIB) 6mg	25,600	204,800*	F-Th-B(IIIB) 6mg	1,600	200
6	Th-B(IIIB) 30mg	51,200	102,400	F-Th-B(IIIB) 30mg	25,600	12,800
7	Th-B(IIIB) 30mg	204,800	102,400	F-Th-B(IIIB) 30mg	25,600	12,800
8	Th-B(IIIB) 30mg	51,200	25,600	F-Th-B(IIIB) 30mg	6,400	12,800
9		51,200	51,200		3,200	6,400
10		12,800	25,600		800	800
11		51,200	25,600		800	1,600
12		51,200	25,600		1,600	800
13		25,600	25,600		200	200
14	Th-B(IIIB) 6mg	51,200	25,600	F-Th-B(IIIB) 1mg	200	400
15		102,400	12,800		800	800
16	Th-B(MN) 6mg	25,600	12,800	Th-B(IIIB) 6mg	100	0
17	Th-B(MN) 6mg	12,800	3,200	Th-B(MN) 6mg	1,600	3,200
18		25,600	6,400		6,400	25,600
19	Th-B(MN) 6mg	25,600	1,600	Th-B(MN) 6mg	6,400	51,200
20		51,200	6,400	Th-B(MN) 6mg	51,200	102,400#

Titers are endpoint ELISA titers (titers at which E/C were ≥ 3.0) against the Th-B peptide, T1-SP10IIIB.
* Animal 1028 did not receive the month 5 injection due to a sterile abscess at the injection site. All injections in animal 1028 after month 5 were in PBS alone.
# Animal 1070 did not receive the month 20 immunization due to the presence of high levels of anti-HIV neutralize antibodies.
For animals 884 and 1028, immunizations at months 2-5 were with T1-SP10IIIB, months 6,7,8 and 14, T1-SP10IIIB(A). For animals 1045 and 1070 immunization at month 16 was with T1-SP10IIIB(A).

Table 10

Mean Lymphocyte and Lymphocyte Subset Levels In Chimpanzees
Before and During Immunization With HIV Envelope Synthetic Peptides*

Leukocyte Subset	Chimpanzee Number											
	884			1028			1045			1070		
	Before	During	% Change	Before	During	% Change	Before	During	% Change	Before	During	% Change
	Cells/mm^3 ± SEM											
Total Lymphocytes	4034±452	3046±249	-26%	3164±396	3286±660	+4%	3164±397	1426±116	-55%†	3943±885	2768±296	-30%
T cells	2629±384	2054±178	-24%	2565±276	2027±402	-21%	2460±253	1012±82	-59%†	3337±762	1887±184	-44%‡
B cells	356±47	365±39	+3%	411±103	458±47	+11%	293±32	175±15	-40%$	302±53	232±22	-23%
NK cells	345±82	317±43	-9%	257±25	434±128	+68%	112±27	61±7	-45%‡	478±148	306±44	-36%

*"Before" samples were studied over a 5 month period prior to immunization with peptides; n = 5 for lymphocytes, n = 3 for T cells, B cells and NK cells. "During" samples were taken from months 2-14 of immunization; n = 11 for lymphocytes, T,B, and NK cells. Unless noted, p values for percent change comparing "before" values with "during" values was not significant with p> .05 using student's t test.

† = p> .001
‡ = p> .02
$ = p> .005

Table #11

Neutralization of HIV LAI/IIIB and HIV MN in Syncytium Inhibition Assay in Chimpanzees Immunized with T1-SP10 Peptides

Animal No.	Month 18		Month 19		Month 20	
	LAI/IIIB	MN	LAI/IIIB	MN	LAI/IIIB	MN
	Presence of Neutralization in Syncytium Inhibition Assay (Reciprocal Titer in RT Inhibition Assay)					
884	-	- (20)	-	-	-	- (24)
1028	-	-	-	-	-	-
1045	-	- (23)	+/- (23)	- (23)	- (22)	- (24)
1070	+/- (92)	- (22)	+ (100)	+ (96)	+/- (86)	++ (350)

- = < 48% inhibition of syncytia.
+/- = ≥ 49% and < 80% inhibition of syncytia.
+ = ≥ 80% inhibition of syncytia, titer 1:10.
++ = ≥ 80% inhibition of syncytia, titer 1:20.

Table 12

Reactivity of Chimpanzee Serum with Truncated Forms of the Th-B Peptide T1-SP10IIIB#

Chimpanzee No. (Bleed Date)	Peptide Used in ELISA Binding Assay				
	T1-SP10IIIB	T1-flu	SP10C	SP10D	SP10E
	Endpoint Titer (> 3.0 E/C) In ELISA Assay				
884 (Month 7)	204,800	800	> 102,400*	51,200	3,200
1028 (Month 7)	102,400	800	102,400	51,200	3,200

#Peptides used in ELISA Assay were:

T1-SP10IIIB	-	KQIINMWQEVGKAMYACTRPNNNTRKSIRIQRGPG
T1-flu	-	KQIINMWQEVGKAMYATYQRTRALVTG
SP10C	-	(C)TRKSIRIQRGPGR(Y)
SP10D	-	(C)IRIQRGPGR
SP10E	-	(C)TRPNNNTRKSIR

ELISA assay performed as described in Methods.
Flu sequence (TYQRTRALVTG) is from influenza nucleoprotein, strain A PR/8/34 from Deres et al, Nature 342:561 (1989).

* at 1:102,400 = 6.0.

Table 13

Effect of Derivatizing T1-SP10IIIB(A) Peptide With the HIV gp41 Fusogenic (F) Domain on Peptide Ability to Bind to Human Cells

Peptide	Antibody	MFC 4 Degrees C, 1 Hr.	MFC 37 Degrees C, 21 Hr.
None	Anti-gp120	7.6	13.6
T1-SP10IIIB(A) 10ug/ml	Anti-gp120	14.7	14.0
F1-T1-SP10IIIB(A)	Anti-gp120	82.8	36.7

Anti-gp120 momoclonal antibody was 0.5beta from the NIAID AIDS Research and Reference Reagent Program (Matsushita et al J. Virol. 62:2107, 1988). Cells used were human JY B cells which were incubated either for 1 hour at 4 degrees C or for 21 hours at 37 degrees C and then reacted with saturating amounts of the anti-gp120IIIB mab, 0.5beta followed by FITC-conjugated goat anti-mouse Ig reagent. The amount of fluoresence was determined on a flow cytometer and fluoresence brightness was expressed as MFC=mean channel fluoresence.

Table shows that conjugation of the F domain on the T1-SP10IIIB(A) peptide confers on it the ability to bind to JY B cells better that the T1-SP10IIIB(A) peptide alone, and that after incubation at 37 degrees C, the F-T1-SP10IIIB(A) peptide is decreased on the surface of the cells.

Table 14

Reactivity of anti-gp120 Monoclonal Antibody with Acetone-Fixed JY B Cells That Had Been Incubated With F-T1-SP10IIIB(A) Peptide (10μg/ml) For 21 Hours at 37 Degrees C

Peptide	Antibody	% Intracytoplasmic Positive
T1-SP10IIIB(A)	Control	0
T1-SP10IIIB(A)	Anti-gp120	0
F-T1-SP10IIIB(A)	Control	0
F-T1-Sp10IIIB(A)	Anti-gp120	76 faint, 24 bright

Cells were incubated as descirbed in Table 13. After 21 hours at 37 degrees C, cytocentrifuge preparations of cells were prepared, acetone fixed, and reacted either with control mab P3x63 Ag8 or with anti-gp120 mab 0.5beta. Slides were read for either faint or bright intracytoplasmic fluoresence on a fluoresence microscope. Data show that after incubation of 10 ug/ml of peptide for 21 hours at 37 degrees C, the F-T1-SP10IIIB(A) peptide could be detected inside the JY B cells whereas the T1-SP10MN(A) peptide could not be detected.

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division

Application deficiencies found during scanning:

☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☑ ***Scanned copy is best available.*** Drawing figure 8 is dark